

CLAIMS

1. An isolated polypeptide having an enzymatic glycosyltransferase activity capable of forming dextrans having $\alpha(1\rightarrow2)$ linkages from saccharose, α -D-fluoroglucose, para-nitrophenyl- α -D-glucopyranoside, α -D-glucopyranoside- α -D-sorbofuranoside or 4-O-
5 α -D-galactopyranosylsucrose, characterized in that it comprises at least one glucan binding domain and a catalytic activity domain located downstream of the glucan binding domain.
2. A polypeptide according to claim 1, comprising at least two catalytic domains located either side of the glucan binding domain.
- 10 3. A polypeptide according to claim 1 or claim 2, comprising a peptide signal, a variable zone, two catalytic domains and a glucan binding domain located between the two catalytic domains.
4. A polypeptide according to one of claims 1 to 3, in which the domain or domains with a catalytic activity has/have a percentage similarity in the range 65% to 100%
15 with SEQ ID No: 1.
5. A polypeptide according to one of the preceding claims, in which the size of the glucan binding domain is more than 500 amino acids.
6. A polypeptide according to claim 5, containing SEQ ID No: 2.
7. A polypeptide according to claim 6, modified by substitution, insertion or deletion of
20 amino acid sequences and comprising sequences having at least 80% and preferably at least 90% similarity with the following sequences of SEQ ID No: 2:

423-439 (SEQ ID No: 6)
478-501 (SEQ ID No: 7)
519-539 (SEQ ID No: 8)
560-571 (SEQ ID No: 9)
631-645 (SEQ ID No: 10)
1014-1021 (SEQ ID No: 11)

2120-2138 (SEQ ID No: 12)
2161-2184 (SEQ ID No: 13)
2202-2214 (SEQ ID No: 14)
2243-2250 (SEQ ID No: 15)
2315-2322 (SEQ ID No: 16)
2689-2696 (SEQ ID No: 17)

8. A polypeptide according to claim 7, in which the following amino acids are unchanged:

W in positions 425 and 2122;

E in positions 430, 565 and 2127, 2248;

5 D in positions 487, 489, 527, 638, 2170, 2172, 2210 and 2322;

H in position 637 and 2321;

Q in position 1019 and 2694.

9. An isolated nucleic acid encoding an enzyme with glycosyltransferase activity that can form dextrans having $\alpha(1 \rightarrow 2)$ linkages from saccharose, α -D-fluoroglucose, para-nitrophenyl- α -D-glucopyranoside, α -D-glucopyranoside- α -D-sorbofuranoside or 4-O- α -D-galactopyranosylsucrose and comprising at least one nucleotide sequence encoding a catalytic domain having at least 50%, preferably at least 80% identity with SEQ ID No: 3, and located 3' of a sequence encoding a glucan binding domain.

10. A nucleic acid according to claim 9, comprising:

- 15 a) two sequences encoding catalytic domains having at least 50%, preferably at least 80% identity with SEQ ID No: 3;
- b) a sequence encoding the glucan binding domain, the latter preferably being located between the two sequences in a).

11. An isolated nucleic acid according to claim 10, having at least 80% identity with:

- 20 a) SEQ ID No: 4; or
- b) the complementary strand to the sequence in a); or
- c) a sequence hybridizing a) or b) under stringent conditions.

12. An isolated nucleic acid according to claim 11, constituted by SEQ ID No: 4 or its complementary strand or the sequence deduced from degeneracy of the genetic code.

- 25 13. An isolated nucleic acid according to claim 11 comprising:

- a) a sequence having at least 80% identity with the sequence encoding a dextranucrase expressed by the plasmid pCR-Ty-dsrD deposited at the CNCM on 15th March 2001 with accession number I-2649; or
- b) A complementary sequence to the sequence in a).

- 5 14. An expression vector comprising a nucleic acid according to any one of claims 9 to 13.
15. A vector according to claim 14, in which the nucleic acid is under the control of a sequence allowing its expression in prokaryotic or eukaryotic cells.
16. A host cell transformed by a nucleic acid according to one of claims 9 to 13 or a
10 vector according to claim 14 or claim 15.
17. A transformed cell according to claim 16, selected from the group comprising *E. coli*, *Leuconostocci*, plants, *Lactococci* and *Bacilli* or yeasts.
18. A transformed cell according to claim 17, characterized in that it is a strain of *E. coli* deposited at the CNCM on 15th March 2001 with accession number I-2649.
- 15 19. A method for producing a dextranucrase that can form $\alpha(1 \rightarrow 2)$ bonds, comprising:
 - a) inserting a nucleic acid according to one of claims 9 to 13 or a vector according to claim 14 or claim 15 into a host cell according to claim 16;
 - b) purifying the enzyme from a cell extract.
20. A method according to claim 19, in which the host cell is a prokaryote selected from
20 a group comprising *E. coli*, *Lactococci*, *Bacilli* and *Leuconostocci*.
21. A method according to claim 19, in which the host cell is a eukaryote selected from a group comprising yeasts, fungi and plants.
22. A method for obtaining a dextranucrase that can form oligosides or dextrans having a percentage of $\alpha(1 \rightarrow 2)$ bonds of more than 30% of the total bonds, comprising a step
25 for modifying SEQ ID No: 4 by addition, deletion or mutation provided that:
 - the reading frame is not modified; and

- the following amino acids are conserved after translation:

W in positions 425 or 2122, encoded by the TGG triplet in positions 1273 and 6364;

E in positions 430, 565, 2127 and 2248, encoded by GAA triplets in positions 1288, 1693, 6379 and 6742 respectively;

D in positions 487, 489, 527, 638, 2170 and 2210, encoded by GAT triplets in positions 1459, 1465, 1579, 1912, 6508 and 6628 respectively;

D in positions 2172 and 2322 encoded by GAT triplets in positions 6514 and 6964;

H in position 637 and 2321, respectively encoded by the CAT triplet in position 1909 and CAC in position 6961;

Q in positions 1019 and 2694, respectively encoded by triplets CAA (position 3055) and CAG (position 8080).

23. A method for obtaining an isolated glycosyltransferase that can form oligosides or dextrans having more than 30% of $\alpha(1\rightarrow2)$ bonds, comprising:

- a step for randomly modifying SEQ ID No: 4 and establishing a library of variations;
- a step for expressing a host housing a variation from said modified sequences in a suitable host cell;
- a step for screening hosts expressing an enzyme that can form more 30% of $\alpha(1\rightarrow2)$ bonds on a suitable substrate;
- a step for isolating the improved gene or genes.

24. A glycosyltransferase that can form at least 30% of $\alpha(1\rightarrow2)$ bonds that can be obtained by a method according to one of claims 19 to 22.

25. Use of a glycosyltransferase obtained by a method according to one of claims 19 to 22 in the production of a composition with a prebiotic effect.

26. Use of a glycosyltransferase obtained by a method according to one of claims 19 to 23 in the production of a pharmaceutical or cosmetic composition.